

**REMARKS**

Applicants are amending their claims in order to further clarify the definition of various aspects of the present invention. Specifically, Applicants have amended claim 6 to recite that the first eluent is supplied to the column, and contaminants in the test solution that are "low in polarity" are eluted; claim 6 has been further amended to recite the additional step of controlling an eluent ratio of the first and second eluents in a gradient mixer and distribution in a fraction collector, by a controller; and to recite controlling the fraction collector to fractionate eluted solution by the controller, when controlling the eluent ratio of the second eluent and the first eluent in the gradient mixer to a predetermined ratio.

In connection with amendments to claim 6, note, for example, pages 10 and 11 of Applicants' specification. Note especially the description in the sole full paragraph on page 10 of Applicants' specification, that with an eluent low in polarity such as n-hexane, contaminants low in polarity are eluted. Note also the third full paragraph on page 9 of Applicants' disclosure, describing a controller 300 (see Fig. 1 of Applicants' disclosure) with a built-in computer, the controller controlling the eluent ratio in the gradient mixer 140, the flow rate in the pump 150 and the distribution and fractions in the fraction collector 180.

Initially, reconsideration and withdrawal of the finality of the Office Action mailed February 27, 2009, is respectfully requested. In this regard, note that while claim 5 was in the application at the time of issuance of the Office Action mailed October 14, 2008, claim 5 was not rejected in this Office Action mailed October 14, 2008. Thus, the first rejection of claim 5 was in the Office Action mailed February 27, 2009. In view of these facts, in particular wherein claim 5 was rejected for the first time in the Office Action mailed February 27, 2009, notwithstanding that

such claim was previously in the application at the time of issuance of the Office Action mailed October 14, 2008, it is respectfully submitted that the Examiner cannot properly make the Office Action mailed February 27, 2009, a Final rejection. That is, as the Office Action mailed February 27, 2009 set forth a first rejection of a claim in the application at the time of original filing, this Office Action mailed February 27, 2009, cannot properly be made a Final rejection.

The contentions by the Examiner in the first full paragraph on page 6 of the Office Action mailed February 27, 2009, are noted. Reference by the Examiner to handling of claim 5 in the Office Action mailed October 14, 2008, being “an obvious, inadvertent typographical error”, is noted. Claim 5 was not set forth on the Office Action Summary or in the detailed portion of the Office Action mailed October 14, 2008; it is not seen how such consistent lack of reference to claim 5 can be considered “an obvious, inadvertent typographical error” (emphasis added).

Applicants respectfully traverse the conclusion by the Examiner that claim 5 is directed to identical subject matter as rejected claim 3. Claim 5 is dependent on claim 2, with claim 3 being dependent on claim 6, claim 2 further defining the solvent and eluents. Clearly the Examiner errs in contending that claim 5 recites the same subject matter as claim 3. See the fourth paragraph of 35 USC 112.

In general, because the Examiner did not set forth a rejection of claim 5 in the Office Action mailed October 14, 2008, the present response constitutes Applicants’ first opportunity to address a rejection of claim 5. In view thereof, clearly finality of the Office Action mailed February 27, 2009, is in error; and reconsideration and withdrawal of finality thereof, with entry of the present amendments as a matter of right, are respectfully requested.

In any event, it is respectfully requested that the present amendments be entered, even where the Finality of the Office Action mailed February 27, 2009, is maintained. In this regard, and as seen in previously referred to portions of Applicants' specification, it is respectfully submitted that the present amendments do not raise any issue of new matter; and it is respectfully submitted that in view of prior arguments, the present amendments do not raise any new issues. As the presently amended claims clearly overcome various of the rejections in the Office Action mailed February 27, 2009, it is respectfully submitted that the present amendments materially limit any issues remaining in the above-identified application. Moreover, noting the various new grounds of rejection in the Office Action mailed February 27, 2009, as compared with the rejections in the prior Office Action mailed October 14, 2008; and also noting the additional arguments by the Examiner in the Office Action mailed February 27, 2009, it is respectfully submitted that the present amendments are clearly timely.

In view of the foregoing, it is respectfully submitted that Applicants have provided the necessary showing under 37 CFR 1.116(b), even where finality of the Office Action mailed February 27, 2009, is not withdrawn; and, accordingly, it is respectfully submitted that entry of the present amendments is clearly proper.

The rejection of claims under the second paragraph of 35 USC 112, as being indefinite, set forth in the first paragraph on page 2 of the Office Action mailed February 27, 2009, is noted. The basis for this rejection is the contention by the Examiner that the step of supplying a first eluent and eluting contaminants "implies that the contaminants are removed by the first eluent", and as such the phrase renders the claims indefinite. However, as is clear from page 10 of Applicants' specification, contaminants "low in polarity" are eluted when introducing the first

eluent. In this regard, note particularly page 10, lines 10-15, of Applicants' specification, describing elution of contaminants low in polarity with the eluent low in polarity such as n-hexane. Clearly, and contrary to the contention by the Examiner, Applicants' specification describes removal of specified contaminants by the first eluent; and, accordingly, the supplying a first eluent and eluting contaminants as in claim 6 does not render the claims indefinite.

Applicants respectfully traverse the rejection of claims 2, 3, 5 and 6 under the first paragraph of 35 USC 112, as failing to comply with the written description requirement. As a first basis for this rejection, in the second paragraph on page 2 of the Office Action mailed February 27, 2009, the Examiner contends that there is no support for "supplying a first eluent . . . and eluting contaminants", the Examiner contending that the first two full paragraphs of page 11 of Applicants' specification "make clear that contaminants are still present at the end of fractionation". Again, the Examiner's attention is respectfully directed to the sole full paragraph on page 10 of Applicants' specification, in particular the description therein that with the eluent low in polarity such as n-hexane, "contaminants low in polarity are eluted". It is respectfully submitted that such description provides sufficient support for the "supplying a first eluent . . . and eluting contaminants", for satisfying requirements of the first paragraph of 35 USC 112.

With respect to the basis for rejection of claims under the first paragraph of 35 USC 112, as set forth in the paragraph bridging pages 2 and 3 of the Office Action mailed February 27, 2009, note that the language "starting fractionating" has been deleted from claim 6, claim 6 now reciting the step of controlling the fraction collector to fractionate eluted solution by the controller, when controlling the eluent ratio of the second eluent and the first eluent in the gradient mixture to a

predetermined ratio. In connection therewith, note the third full paragraph on page 9 of Applicants' specification. It is respectfully submitted that claim 6 as presently amended is clearly supported by the written description in Applicants' specification, especially on page 9 thereof, so as to clearly overcome the rejection as set forth in the paragraph bridging pages 2 and 3 of the Office Action mailed February 27, 2009.

Applicants respectfully submit that all of the claims presented for consideration by the Examiner on the merits, that is, claims 2, 3, 5 and 6, patentably distinguish over the teachings of the references applied by the Examiner in rejecting claims formerly in the application, that is, the teachings of U.S. Patent No. 5,076,909 to Overfield, et al., the Japanese patent documents to Matsushita, et al., No. 56-89058, and to Takamura, No. 6-94697, and English translations thereof, and the publication by Snyder, Introduction to Modern Liquid Chromatography (1979), pages 662-686, under the provisions of 35 USC 103.

It is respectfully submitted that the teachings of the references as applied by the Examiner would have neither disclosed nor would have suggested such a method for fractionating polycyclic aromatic hydrocarbons using (a) a test solution in which a sample containing polycyclic aromatic hydrocarbons is dissolved in a solvent, (b) a plurality of eluents to be a mobile phase for the test solution and different from the solvent in which the sample is dissolved and different in polarity from each other, and (c) a column packed with a packing material, and wherein the method includes, inter alia, supplying a first eluent to the column and eluting contaminants low in polarity in the test solution; mixing gradually a second eluent which is higher in polarity than the first eluent while reducing flow of the first eluent; controlling an eluent ratio of the first and second eluents in a gradient mixer, and a distribution in a fraction collector, by a controller; and controlling the fraction collector

to fractionate eluted solution by the controller, when controlling the eluent ratio of the first and second eluents in the gradient mixer to a predetermined ratio. See claim 6.

That is, as will be discussed in more detail infra, according to the present invention, steps of controlling the eluent ratio in a gradient mixer and the distribution in a fraction collector, by a controller, and of controlling the fraction collector to fractionate eluted solution, by the controller, when controlling the eluent ratio of the first and second eluents in the gradient mixer to a predetermined ratio, are performed. It is respectfully submitted that these applied references do not disclose, nor would have suggested, such controlling steps as in the present claims, using the controller, and advantages achieved thereby wherein a target substance or substances (e.g., nitropolycyclic aromatic hydrocarbons and polycyclic aromatic hydrocarbons other than the nitropolycyclic aromatic hydrocarbons) can be quantitatively analyzed in a relatively simple and effective manner, and accurately.

Moreover, and as will also be discussed further infra, it is respectfully submitted that the combined teachings of the applied references would have neither disclosed nor would have suggested, in such a method for fractionating polycyclic aromatic hydrocarbons as in the present claims, including the controlling steps in claim 6, and among other features therein, the gradual mixing of a second eluent which is higher in polarity than the first eluent while reducing flow of the first eluent, and, moreover, wherein the first eluent elutes contaminants low in polarity in the test solution.

Furthermore, it is respectfully submitted that the teachings of the applied references would have neither disclosed nor would have suggested such fractionating method as in the present claims, having features as discussed previously in connection with claim 6, and, additionally, wherein the solvent in which

the sample containing polycyclic aromatic hydrocarbons is dissolved in an alcohol, one of the plurality of eluents includes dichloromethane, and an eluent lower in polarity than dichloromethane includes any one of n-hexane, carbon tetrachloride and toluene (see claim 2); and/or wherein the column is a silica gel column (see claims 3 and 5).

The present invention as claimed in the above-identified application is directed to a method for fractionating polycyclic aromatic hydrocarbons; this method can be used in connection with analysis of polycyclic aromatic hydrocarbons occurring in exhaust gas microparticulates contained in the atmosphere or in engine exhaust gas. The method claimed in the above-identified application can be utilized for fractionation of nitropolycyclic aromatic hydrocarbons, and, subsequently, quantitatively analyzing the nitropolycyclic aromatic hydrocarbons and polycyclic aromatic hydrocarbons other than the nitropolycyclic aromatic hydrocarbons, respectively.

Polycyclic aromatic hydrocarbons and nitropolycyclic aromatic hydrocarbons contained in a solvent-soluble organosoluble fraction (SOF) in exhausts from diesel gas have been analyzed, although their contents are extremely small, because of their high carcinogenicity. Because the contents of these substances are small, these substances cannot be detected unless a large number of interfering substances are eliminated and a pretreatment is performed, prior to analysis, to enhance the analytical sensitivity and precision. Due thereto, the sample to be tested is required to be subjected to a preanalysis treatment to eliminate interfering components in such a way that the sample adapts to the targets to be analyzed.

Various procedures for quantitative analysis of the polycyclic aromatic hydrocarbon have been proposed, as described on pages 3 and 4 of Applicants'

specification; these proposed techniques include a third step of purification, which is a step of fractionating nitropolycyclic aromatic hydrocarbons or polycyclic aromatic hydrocarbons other than the nitropolycyclic aromatic hydrocarbons, by various techniques as described in the first full paragraph on page 4 of Applicants' specification.

However, the previously proposed techniques for the quantitative analysis have disadvantages, including the requirement of a large number of steps and a long operation time, wherein it is highly probable that fractionated substances are lost or decomposed; and there are also problems including a reproducibility problem, as described on pages 4-6 of Applicants' specification.

Against this background, Applicants avoid disadvantages in previously proposed techniques, providing a method for fractionating polycyclic aromatic hydrocarbons, useful in the aforementioned purification step, which can efficiently and effectively separate the nitropolycyclic aromatic hydrocarbons, the polycyclic aromatic hydrocarbons other than the nitropolycyclic aromatic hydrocarbons, and the interfering substances from the SOF. Specifically, according to the present method, utilizing a column packed with a packing material and a plurality of eluents to be a mobile phase for the test solution, a first eluent is supplied to the column and contaminants low in polarity, in the test solution, are eluted; a second eluent which is higher in polarity than the first eluent is mixed gradually while reducing flow of the first eluent; the eluent ratio in a gradient mixer and distribution in a fraction collector are controlled by a controller; and the fraction collector is controlled by the controller to fractionate eluted solution, when controlling the eluent ratio of the second eluent in the gradient mixer to a predetermined ratio. According to the present invention,



interfering components can be eliminated from the target substance to be analyzed, in a relatively simple and effective manner, and an accurate analysis is achieved.

Thus, according to the present invention, the controller controls the fraction collector and the eluent ratio, to fractionate when the eluent ratio of the second eluent to first eluent increases and reaches a predetermined ratio. The controller controls to start fractionation when the eluent ratio reaches a predetermined ratio, whereby the present invention advantageously achieves an efficient separation of components, such that accurate analysis can be achieved.

Matsushita, et al. discloses a chromatographic analytic method for hydrocarbon compounds that separates hydrocarbon compounds contained in a solution into three components, namely, saturates, unsaturates and aromatics, by means of high-performance liquid chromatography. The method includes use of an organochlorine solvent or a mixture of an organochlorine solvent and hydrocarbon solvent as an eluent; a first column that is filled with a silica-based filler; and a second column that is filled with a silver-containing silica-based filler, the two columns being connected in series, the first column serving to separate the hydrocarbon compounds into (saturates and unsaturates) and aromatics, and the saturates, unsaturates and aromatics in this order being eluted from the second column, without substantially affecting the separation of the components eluted from the first column, and these being introduced into a detector and detected quantitatively. Note the sole full paragraph on page 4 of the English translation of this patent document. See also the third full paragraph on page 5 and the first full paragraph on page 6, of this patent document.

It is respectfully submitted that Matsushita, et al. utilizes two columns, for providing the separation. It is respectfully submitted that this reference does not

disclose, nor would have suggested, use of a plurality of eluents to be a mobile phase for the test solution, the plurality of eluents being different from the solvent in which the sample is dissolved and differing in polarity from each other, with a first eluent being supplied to the column and eluting contaminants low in polarity; and a second eluent being mixed gradually which is higher in polarity than the first eluent while reducing flow of the first eluent; and the controlling using the controller, and advantages due thereto.

As can be seen in the foregoing with respect to Matsushita, et al., and also with respect to the teachings of Takamura, these references do not disclose, nor would have suggested, use of the plurality of eluents respectively to elute specified contaminants and the polycyclic aromatic hydrocarbons, with the recited gradual mixing of the second eluent higher in polarity than the first eluent while reducing flow of the first eluent; and with (a) the controlling of the eluent ratio in the gradient mixer and the distribution in the fraction collector, and (b) the controlling the fraction collector to fractionate eluted solution when controlling the eluent ratio in the gradient mixer to a predetermined, each by a controller, as in the present claims.

Takamura discloses an analytical method including passing a hydrocarbon oil to a first column and separating it into a saturated portion containing an olefin portion and an aromatic group portion by using a liquid chromatograph; applying a backflush to the first column after the saturated portion is eluted from the first column; passing the saturated portion eluted from the first column through a silver ion-carrying second column and separating it from the olefin portion; detecting the saturated portion from the silver ion-carrying second column by a differential refractometer and fractionating it; detecting the peak of the saturated portion and applying a backflush to the silver ion-carrying second column; detecting the olefin portion eluted from the

silver ion-carrying second column by a differential refractometer and fractionating it; detecting the peak of the olefin portion and re-applying a backflush to the silver ion-carrying second column; and detecting the aromatic portion eluted from the silver ion-carrying second column by the differential refractometer and fractionating it. Note, for example, section [0016] on pages 10 and 11 of the English translation of Takamura.

As can be appreciated from the foregoing, Takamura has a relatively complex analysis procedure utilizing back-flushing and two columns, including a silver ion-carrying second column. It is respectfully submitted that the teachings of this reference do not disclose, nor would have suggested, the method as in the present claims, including steps discussed previously such as supplying the first eluent to the column and eluting contaminants low in polarity; and mixing gradually the second eluent which is higher in polarity than the first eluent while reducing flow of the first eluent, with the controlling steps, and advantages thereof.

It is respectfully submitted that the additional teachings of Snyder and of Overfield, et al. would not have rectified the deficiencies of either of Matsushita, et al. or of Takamura, such that the presently claimed invention as a whole would have been obvious to one of ordinary skill in the art.

Snyder, as applied by the Examiner, describes gradient elution, disclosing that gradient elution devices provide for the admixture of two (or more) solvents, a weak initial solvent A and a strong solvent B, so that the concentration of B in the mobile phase entering the column increases throughout the separation; and, as a result, the mobile phase provides large  $k'$  values for the sample of interest at the beginning of separation and small  $k'$  values at the end of separation, that is, the

solvent is initially weak and becomes progressively stronger as separation proceeds.  
See page 664 of Snyder.

Overfield, et al. discloses a method for the spectroscopic analysis of solutions containing at least two aromatic compounds for the aromatic core content, including steps of irradiating the solution with UV light having a wavelength range of which at least a portion is within the range of about 200 nm-500 nm; measuring absorbance; deriving the integral of absorbance as a function of photon energy; and comparing the absorbance integral to a predetermined value, to thereby obtain the aromatic core content. As applied by the Examiner, note from column 19, line 18, through column 20, line 59, of this patent. This disclosure includes a description that, initially, a weak solvent is employed which is substantially transparent to UV radiation and has a sufficient solubility parameter to dissolve all components of the sample to be introduced into the column but of which the solubility parameter is not so high that relatively sharp discrimination between different chemical types in the sample by high performance liquid chromatography will not be possible; during a first period of operation a sample of the hydrocarbon which is to be analyzed is passed to a location where it co-mingles with a weak solvent to form a substantially uniform solution which is free of precipitated materials such as asphaltenes; and, subsequently, when substantially all the saturated and aromatic hydrocarbon molecules have been eluted from the column by the weak solvent, as evidenced by a decline in the UV absorption and refractive index to virtually their base values with the solvent only, a strong solvent (that is, a solvent having relatively high polarity) is passed into the column at a progressively increasing rate while the weak solvent is pumped at a correspondingly progressively reducing rate so that the total volume-rate of solvent is substantially unaltered, and after a selected third time period the

weak solvent is totally absent and the only solvent passing to the upstream end of the column is the strong solvent.

Even assuming, arguendo, that the teachings of Snyder and of Overfield, et al. as applied by the Examiner were properly combinable with the teachings of either of Matsushita, et al. or of Takamura, the combined teachings would have neither disclosed nor would have suggested such features of the present invention as discussed previously, including the supply of first eluent and eluting contaminants low in polarity; and mixing gradually the second eluent higher in polarity than the first eluent while reducing flow of the first eluent, with (a) control of the eluent ratio in the gradient mixer and of distribution in the fraction collector, by a controller, and (b) control of the fraction collector to fractionate eluted solution, by the controller, the latter control being performed when controlling the eluent ratio in the gradient mixer to a predetermined ratio, as in claim 6; and/or additional features of the present invention as in the other claims being considered on the merits herein.

Contentions by the Examiner in the paragraph bridging pages 3 and 4 of the Office Action mailed February 27, 2009, with respect to differences between each of the primary references (Matsushita, et al. and Takamura) and the previously considered claims, are noted. However, it is respectfully submitted that the presently claimed invention differs from the teachings of these references by much more than the recitation of increasing the polarity of the eluents; and it is respectfully submitted that the teachings of the primary references, even in combination with the teachings of the secondary references as applied by the Examiner, would have neither disclosed nor would have suggested supply of the first eluent and eluting contaminants low in polarity, with the mixing of the second eluent and the controlling steps using the controller, as in each of the present claims, and advantages thereof.

The contention by the Examiner in the paragraph bridging pages 5 and 6 of the Office Action mailed February 27, 2009, that the first two full paragraphs of page 11 of the specification make clear that contaminants are present from the beginning of the elution “and remain at the end of fractionation” is noted. However, as is clear from page 10 of Applicants’ specification, contaminants low in polarity are eluted with the first eluent. Clearly the Examiner errs if interpreting the present invention as having all contaminants remaining at the end of fractionation.

The contention by the Examiner in this paragraph bridging pages 5 and 6 of the Office Action mailed February 27, 2009, that “the claims are merely directed to gradient elution”, is respectfully traversed. It is respectfully submitted that the claims recite much more than gradient elution, reciting the elution of contaminants low in polarity in the test solution with supplying the first eluent to the column; and, moreover, controlling the eluent ratio in the gradient mixer and the distribution in a fraction collector by a controller, and controlling the fraction collector to fractionate eluted solution, by the controller, when controlling the eluent ratio of the second eluent and the first eluent in the gradient mixer to a predetermined ratio. It is respectfully submitted that the teachings of the applied references would have neither disclosed nor suggested such features of the present invention, and advantages achieved thereby; or, together with such features, the additional features in the dependent claims, and advantages due thereto.

In view of the foregoing comments and amendments, reconsideration and withdrawal of the finality of the Office Action mailed February 27, 2009, with a corresponding entry of the present amendments, and reconsideration and allowance of all claims being considered on the merits, then in the application, are respectfully requested.

In any event, entry of the present amendments, and reconsideration and allowance of all claims then in the application, being considered on the merits, are respectfully requested.

To the extent necessary, Applicants hereby petition for an extension of time under 37 CFR 1.136. Kindly charge any shortage of fees due in connection with the filing of this paper, including any extension of time fees, to the Deposit Account of Antonelli, Terry, Stout & Kraus, LLP, Account No. 01-2135 (case 648.46078X00), and please credit any overpayments to such Deposit Account.

Respectfully submitted,

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